bryology *of the* Chick *and the* Pig

LABORATORY OUTLINES

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FOR THE STUDY OF THE

Embryology *of the* Chick *and the* Pig

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INTRODUCTION

These laboratory outlines are published as the simplest way of preparing them for the use of my own class in the University of Chicago, with the hope that they may prove of use to others, and may perhaps contribute toward the establishment of a standard of embryological work for medical students. The medical student is required to complete in the laboratory Part I, sections I, III, IV, and all of Part II. To accomplish this much in the seventy or eighty hours at his disposal it is necessary that most of the work of preparation be done for him.

It is perhaps unnecessary to say that this pamphlet is intended to be a guide to the order of study in the laboratory only. A student who did not go through a systematic course of study with lectures or reading at the same time would gain a very imperfect idea of the subject.

METHODS

The methods of study employed in the following outlines are: (1) Study of the living embryo. (2) Study of the entire embryo, (*a*) as an opaque object with the dissecting microscope; (*b*) with the compound microscope, after killing, hardening, staining, clearing, and mounting. (3) Study of embryos by dissection, especially in later stages. (4) Study of serial sections with the microscope.

I. STUDY OF THE LIVING EMBRYO OF THE CHICK

This is best performed under warm normal salt solution (0.75 per cent. NaCl heated to about 38° C.). The egg is opened by gradually picking away the shell at the broad end. The egg membrane is then stripped off. When enough is removed, invert the open end of the egg beneath the salt solution and allow the contents to flow out (note that *the yolk must not be broken*). The embryo may now be examined on the surface of the yolk, as the blastoderm almost invariably turns up, or may easily be made to do so. The blastoderm can now be separated by cutting around it *outside the area vasculosa* with fine scissors. It should then be gently floated into a watch crystal with a flat bottom *submerged* in the

salt solution. The crystal and its contents may then be lifted out. The *vitelline membrane,* a delicate transparent membrane covering the blastoderm, must next be removed. Details for study are given under directions for various stages.

II. PREPARATION OF EMBRYOS FOR ENTIRE MOUNTS AND FOR SECTIONS

1. *First process—killing.*

a) For chick embryos up to 96 hours remove the blastoderm under salt solution, as directed. Spread the blastoderm out flat in the watch crystal. Draw off as much salt solution as possible with a pipette. Let stand for a short time until the edges of the blastoderm adhere slightly. Then add the killing fluid very slowly with a pipette, dropping it on the center of the blastoderm.[1]

b) Older embryos of the chick and embryos of the pig are simply submerged with membranes intact in a bountiful supply of the killing fluid.

c) Killing fluids.

(1) Kleinenberg's picrosulphuric acid (saturated solution of picric acid plus 2 per cent. sul-

[1] In the early stages of the chick embryo the strain produced on the embryo by this method causes more or less dislocation of the mesoblastic somites. This can be avoided only by immersing the entire yolk in a large quantity of the killing fluid and removing the blastoderm with great care after hardening in alcohol.

phuric acid; to this add twice its volume of water). Chick embryos of 24, 36, and 48 hours should be left in at least 1½ and not more than 6 hours; 60, 72, and 96 hour chick embryos and embryos of the pig, 2½-6 hours. After removing the killing fluid, add 70 per cent. alcohol and change daily (oftener at first) until the color ceases to come out of the embryos. Preserve in 80 per cent. alcohol.

(2) Picrosulphuric-acetic acid (Kleinenberg's picrosulphuric acid plus 5 per cent. of glacial acetic acid). This fluid gives a beautiful fixation either for entire mounts of for sections. Use same as (1).

(3) Sublimate-acetic (saturated aqueous solution of corrosive sublimate plus 5 per cent. glacial acetic acid; to give the best results this fluid must be freshly made up). Use like Kleinenberg's fluid, but after removing, add *water,* and change several times. In one hour replace with 35 per cent. alcohol; in about an hour more, 50 per cent.; in yet an hour, 70 per cent., colored with iodine. This last fluid must be changed as long as it continues to be decolorized by extraction of the mercuric chloride, which forms HgI_2. Preserve in 80 per cent. alcohol.

These killing fluids are selected because of the ease with which they can be used. But any one

of a large number of killing fluids might be used with good results.

2. *Second process—staining.*—The methods of staining vary somewhat according to whether the embryo is intended for sectioning or for mounting entire.

a) *For whole mounts.*—Prepare one each of the stages preserved as a permanent whole mount. Chick embryos of more than 96 hours and pig embryos of more than 10 mm. do not make satisfactory whole mounts. Transfer the embryos successively from 80 per cent. to 70 per cent., to 50 per cent., to 35 per cent. alcohol and finally to water. The time in each fluid may be the same for any embryo, but should be rather longer for the larger embryos. Leave the younger embryos about 30 minutes in each fluid; the larger, 45-60 minutes.

For staining: (1) Use Conklin's picro-hæmatoxylin (Delafield's hæmatoxylin diluted with four times its volume of water; to each 6 c.c. of the above add one drop of Kleinenberg's undiluted picrosuphuric acid) and leave in until the embryos are stained through—1–3 hours. (2) Wash in water. (3) Pass up the series of alcohols, 30 minutes or more in each, to 70 per cent. alcohol. (4) Extract the superfluous stain with acidulated alcohol (1 per cent. hydrochloric acid

in 70 per cent. alcohol. (5) Wash repeatedly in 70 per cent. alcohol to get rid of the acid; then transfer to 80 per cent. alcohol and leave in this for several hours for complete removal of the acid; then transfer to 95 per cent. alcohol for 30 minutes. (6) Absolute alcohol for at least one hour, changing at the end of 45 minutes. (7) Introduce a layer of oil of cloves (xylol, turpentine, or cedar oil will do equally well) beneath the alcohol; after the embryos have sunk in the oil and begin to appear transparent remove the fluids and add fresh oil. (8) Mount in balsam, supporting the coverslip so that it does not rest on the embryo.

b) *For sections.*—Stain in borax carmine. The methods are very similar. Note the following *exceptions* to the above rules: (1) Put the embryo into the stain from 50 per cent. alcohol. (2) Leave in at least 12 hours. (3) After staining, wash in 50 per cent. alcohol, and transfer to 70 per cent. acidulated alcohol (for about 6 hours). (4) After clearing in oil, put in melted paraffin for 1–2 hours depending on the size of the embryo. (5) *Imbed.* A deep "embryological" watch-glass is most convenient for imbedding. Smear the sides very lightly with glycerin, pour in melted paraffin; place the embryo in position, and, after the surface of the paraffin

has set, plunge the whole beneath cold water or, better, alcohol. Hardening under alcohol (70 or 80 per cent.) gives the paraffin a beautiful consistency for cutting.

Embryos may be stained in other ways before imbedding Or they may be imbedded *unstained,* and the staining done after sectioning. By the latter method more precise results may be obtained.

3. *Third process—scctioning.*—The use of the microtomes will be demonstrated.

The following outlines are meant to be a guide only to the order of study. For further assistance use Foster and Balfour's *Elements of Embryology,* Duval's *Atlas,* Minot's *Laboratory Text-Book,* and other texts.

PART I

Study of Chick Embryos

STUDY OF CHICK EMBRYOS

I. EMBRYO WITH FROM TEN TO FOURTEEN SOMITES (TWENTY-NINE TO THIRTY-FOUR HOURS)[1]

1. *The egg.*—While opening the egg, observe that the *shell-membrane* is double. The *air-chamber* at the broad end is between its two layers. After pouring the egg into the bowl, observe the twisted denser cords of albumen in the "white" (*chalazae*). Are they attached to the yolk? What function may they have? The *yolk* or *vitellus* is the true *ovum,* the other parts being merely *envelopes.* Observe the *vitelline membrane* containing the yolk.

2. *The living embryo.*—How much of the yolk is covered by the *blastoderm?* In the center of the blastoderm is a transparent slipper-shaped area (*area pellucida*), in which the embryo appears as a narrow white streak running through

[1] This stage has been selected for starting because we have found that the beginner does not readily understand stages prior to the formation of the embryo, in which, moreover, the technique is more difficult. On the other hand, later stages are too complex, and the student is apt to be confused by the flexures. The stage selected is a good starting-point for the study of both younger and older stages.

the center. The part of the blastoderm external to the pellucid area is known as the *area opaca.* How much of the latter is occupied by the *area vasculosa?*

Preserve the blastoderm according to directions given in the introduction.

3. *Entire mount including the vascular area*

a) In the vascular area observe the irregular deeply stained masses (*blood-islands*). At this stage it will be found that they are enclosed in wide anastomosing tubes, the *extra-embryonic blood-vessels,* that open peripherally into the bounding *sinus terminalis.* How are the extra-embryonic connected with the embryonic blood-vessels?

b) The embryo.—Make a careful drawing of the embryo and *area vasculosa.* State whether your drawing is from the *upper* or from the *under* side.

The following structures can be readily identified (others will be seen, but are better understood after study of sections):

(1) The *neural tube* forms the axis of the embryo. In its anterior region identify *fore-brain, optic vesicles, mid-brain, hind-brain.*. The secondary subdivisions of the hind-brain are the *neuromeres.* The portion of the neural tube back of the hind-brain (*cord, myelon*) is closed in front, but open behind.

(2) The *primitive streak* is enclosed by the diverging folds of the myelon.

(3) The *head-fold.* The head of the embryo projects freely above the blastoderm. The fold uniting the ventral surface of the head with the blastoderm is known as the "head-fold."

(4) The *mesoblastic somites,* appearing on each side of the neural tube. Number? The series is continued behind by the undivided *segmental plate.*

(5) The *heart* lies beneath the hind-brain in a special portion of the body-cavity bounded in front by the head-fold and behind by the diverging limbs of the *splanchnopleure.* Its posterior (venous) end receives the *vitelline veins* from the vascular area. Its anterior (arterial) end is prolonged into the *ventral aorta.* Its axis is somewhat bent at this stage. In what direction?

(6) The *head-fold* of the *amnion* is beginning to fold over the anterior end of the head in the older embryos of this stage.

4. *Study of transverse sections.*—The embryo should be cut into a complete series of transverse sections, and these mounted in order. For our purposes sections should be of the uniform thickness of about 20 μ (*i. e.,* 20 micro-millimeters, or 0.020 mm.). The number of any section in the series, therefore, in comparison with the entire

number, will enable the student to ascertain its position with relation to the entire embryo. A scale of the total number of sections should be put at one side of the drawing of the entire embryo. The position of each section may then be determined by its number in the series, provided the proportions of the drawing are correct.[2]

Study each section drawn under the high power as well as under the low power. Some drawings of parts of sections should be made with the high power to show the characters of the individual cells. For low-power drawings use colors to distinguish ectoderm (blue), mesoderm (red), and entoderm (yellow), and do not draw in cells.

Make careful drawings of transverse sections through the following regions:

a) Optic vesicles.
b) Mid-brain.
c) Posterior half of the heart.
d) Myelon in the closed region.
e) Myelon in the open region.
f) Through the primitive streak.

[2] This will work out with perfect accuracy only in case the sections are of an embryo actually drawn entire with the camera lucida. But if the drawing of the entire embryo is correct as to proportions, and the sections are from an embryo of corresponding age, the position of the sections may be determined with considerable accuracy.

Identify and label all the parts with their proper names.

It will be best to draw *d*) first. The following description of this section will aid in the study of the others:

d) The section is bounded dorsally by a very thin cellular membrane, the *ectoderm;* ventrally its boundary is formed by the *entoderm.* The large *neural tube* lies just beneath the ectoderm in the middle line. Beneath the neural tube is a section of the solid, rod-like *notochord.* The mesoderm lies between the parts already named. It consists on each side of the middle line of the following parts: (1) the *mesoblastic somite,* a block of cells that radiate from the common central point or cavity; (2) the *intermediate cell mass* (*nephrotome*) between (1) and (3); (3) the *lateral plate,* split into two layers, one of which (*somatic layer*) is applied to the ectoderm, and the other (*splanchnic layer*) to the entoderm. The space between the two layers of the lateral plate forms the body-cavity (*coelome*). The double membrane formed of the ectoderm and somatic layer of mesoderm is known as the *somatopleure.* The entoderm and splanchnic layer together form the *splanchnopleure.* The two large blood-vessels immediately beneath the somites are the *dorsal aortae.* Note the numer-

ous blood-vessels in the splanchnopleure; compare the somatopleure in this respect.

a) This section, being taken in front of the head-fold, shows the head free from the blastoderm. Is the ectoderm in contact with the optic vesicles thickened? The mesoderm is represented by a few anastomosing branching cells (*mesenchyme*). The section is in front of the anterior end of the fore-gut, and so the alimentary tract does not appear. Is entoderm present? Where?

b) In this region the splanchnopleure has folded so as to form a tube, the beginning of the alimentary tract. The part in the region of the mid- and hind-brain is known as the *pharynx*. What do the lateral expansions in this region signify? The *dorsal aortae* appear here. What is their origin?

c) Trace the parts shown in *b*) back to this section. The heart is the principal part requiring description. It is formed of the splanchnopleure and is attached by a dorsal mesentery (*mesocardium*) to the under wall of the pharynx. Note its two layers:—*muscular* (*myocardium*) and *endothelial* (*endocardium*). The cavity in which it is situated (*pericardium*) is an enlarged part of the general body-cavity.

e) and *f*) require no separate description. Do you find the notochord in *f*)? Where between *c*) and *f*) did it disappear? Draw carefully.

g) Description of parts from study of sections:

(1) Describe the relations of the three germ-layers (ectoderm, mesoderm, and entoderm) in the primitive streak.

(2) Prepare a diagram showing the anatomy of the heart and main blood-vessels.

(3) Describe the form of the intestine. How far back is it closed? How is the *alimentary tube* formed from the splanchnopleure? How is the *neural tube* formed from the *medullary* (*neural*) plate?

(4) Where does the notochord end anteriorly? posteriorly?

h) In the older embryos of this stage the future *auditory pit* is represented by a small disk-shaped thickening of the ectoderm opposite the middle of the hind-brain. Look for this.

5. *Study of sagattal sections.*—Study of this series should confirm and supplement the knowledge gained by study of the transverse series. The sagittal series is particularly valuable for the study of the *head-fold,* the *proamnion,* the *incipient pericardium,* and the *heart.*

II. EMBRYO WITH FROM TWO TO SIX SOMITES (TWENTY TO TWENTY-FOUR HOURS)

1. In the fresh egg observe and describe the relations of the embryo, pellucid area, opaque

area. How much of the yolk is covered by the blastoderm? Compare I.

2. Make a drawing of an entire mount of this stage. Note particularly the condition of the medullary plate. Is the *tube* formed at any place? Can the heart be seen? How far back does the head fold extend? Is the primitive streak longer or shorter than in I?

3. *Sections.*—Draw the following transverse sections:

a) Through the point of divergence of the walls of the fore-gut.

b) Through the somitic region.

c) Through the primitive streak.

Study the formation of the heart, fore-gut, head-fold, etc.

III. EMBRYO WITH FROM TWENTY FOUR TO TWENTY NINE SOMITES (FORTY FOUR TO FORTY-EIGHT HOURS)

1. *Living embryo.*—Describe carefully the changes visible to the naked eye since the thirty-fourth hour. Remove the embryo with the entire vascular area, and preserve it.

2. *Entire mounts*—The most striking changes concern the region of the head. By more rapid growth of the dorsal surface the head has become bent (*cephalic flexure*) in the region of the mid-brain, so that the fore-brain and part of the

mid-brain form almost a right angle with the rest of the head. Moreover, the head has become so far free from the blastoderm, and so compressed laterally, that it now lies on its side (which side?). The dorsal side of the trunk, on the other hand, is still turned up, so that there is a twisting of the axis of the embryo just back of the heart. The tail-fold is not yet formed, or has just begun.

a) *The optic vesicles* are relatively smaller in relation to the brain than in I (not actually smaller, of course). To what part of the fore-brain are they attached? In each distinguish *inner* and *outer layers* of the *retina,* the *lens,* the *choroid fissure,* and the *cavity of the vitreous humor.*

b) The *auditory vesicles* are now closed (?) sacs.

c) The heart has grown greatly in length, and, its two ends being fixed, it has become doubled on itself. What is the relation of the heart to the main afferent and efferent blood-vessels?

d) Two or three *visceral pouches* are now visible. They may be found ventral to the region of the hind-brain. The first or *hyomandibular* pouch is bounded in front by the *first visceral* or *mandibular,* and behind by the *second*

visceral or *hyoid arch;* the second pouch is bounded in front by the *hyoid,* and behind by the *third visceral arch;* the third pouch is bounded in front by the *third visceral arch,* and behind by the *fourth.*

e) How many mesoblastic somites are there? What is the condition of the mesoblastic segmental plate?

f) How far back is the fore-gut closed?

g) Find the head-fold of the amnion. How far back is the amnion closed?

h) What changes have taken place in the vascular area?

Make a drawing of the entire embryo illustrating all of the above points.

3. *Sections.*—A series cut transversely to the trunk will pass horizontally through the fore- and mid-brain (*cf.* drawing of entire embryo).

a) Draw a section through the trunk a short distance behind the heart. Identify and label parts. Note (1) the elevation of the axis of the body, and the way in which the lateral folds (*lateral limiting sulci*) in the somatopleure delimit the embryonic from the extra-embryonic area; (2) appearance of *mesenchyme;* (3) approximation of the two dorsal aortae; (4) the appearance of the *amniotic folds;* (5) in the mesoderm identify *posterior cardinal veins* and

the myotome (muscle-*plate*). The mass of mesenchyme betwen the myotome, on the one hand, and the neural tube and notochord, on the other, is known as the *sclerotome.* What has become of the intermediate cell-mass (nephrotome)? Do you find the Wolffian duct? Note the folding of the splanchnopleure.

Draw also the following sections:

b) Through the optic vesicles: What change in these? Do you find the beginning of the *lens?* What other structures appear in this section? (Refer to the drawing of the entire embryo.) What are the diverticula of the pharynx? Identify the *closed amnion* and the *serosa* (*chorion*).

c) Through the auditory pit, showing fusion of the gill-pouches with the ectoderm. Identify blood-vessels, etc.

d) Through the center of the heart.

e) Through the roots of the vitelline veins.

f) About half-way between *a*) and *g*).

g) Through the primitive streak (if present).

4. The study of this series shows by how much the development of the anterior end is in advance of that of the posterior end. The *tail-fold* is not yet formed or has just begun. As we pass from behind forward, each successive mesoblastic somite shows an advance in development over its immediate predecessor. *On the basis of*

this, describe the origin of the myotome and sclerotome. What becomes of the *intermediate cell mass?* New mesodermic somites are continually being cut off from the anterior end of the segmental plate as long as it lasts.

Write a description of the pharynx in this stage, basing your account, so far as possible, on the preparations studied, and, where necessary, on the models of this stage.

Write a description of the circulation at this stage of the development, illustrating it with a diagram built up from your study of the whole mount and the sections. Do you find any valves in the blood-vessels?

IV. STUDY OF AN EMBRYO WITH CERVICAL FLEXURE FORMED (SIXTY-EIGHT TO SEVENTY-TWO HOURS)

1. *Living embryo.*—Describe carefully the changes visible to the naked eye since the forty-eighth hour. Draw carefully the blood-vessels of the vascular area. Name the arteries and veins. Compare with the figure on p. 111 of Foster and Balfour. Observe the beating of the heart. May the beat be renewed, after stopping, by warming? Preserve the embryo.

2. *Entire mount.*—A second flexure (*cervical* or *nuchal*) has appeared in the head. The *tail-fold* is well formed. What is the position

of the embryo on the blastoderm? Is the amnion completely closed? Observe the appearance of an extension of the primary fore-brain, bilobed anteriorly, the *telencephalon,* rudiment of the cerebral hemispheres; on the ventral surface of the head, a short distance in front of the optic stalks, find the *olfactory pits.* The optic stalks are attached to the floor of the thalamencephalon (*diencephalon*) near the anterior end. The depressed region behind the optic stalks is the *infundibular region.* The *epiphysis* is a short diverticulum of the roof of the *thalamencephalon.* The *mid-brain* (*mesencephalon*) forms the apex of the cranial flexure. It is united to the hind-brain by the narrow *isthmus.* The most anterior division of the hind-brain with thick roof is the *metencephalon* (rudiment of the *cerebellum*). The remainder of the hind-brain with thin transparent roof and sides is the *myelencephalon* (rudiment of the *medulla oblongata*). In the *optic cup* observe the inner and outer walls, the *lens,* the *choroid* fissure, and the cavity of the vitreous humor (posterior chamber). What is the form of the *auditory sac* (*otocyst*)? Above which *visceral arch does it lie?* How many *visceral clefts are there?* The tissue bounding the clefts is thickened to form the *visceral arches.* These are named as follows:

(1) *Mandibular* or *first,* in front of the first cleft (this pair will form the lower jaw); do you find a *maxillary process* arising from the dorsal angle of the arch? (2) *Hyoid* or *second,* behind the first cleft, (3), (4), and (5), the *third, fourth,* and *fifth* visceral arches. The first visceral cleft is also known as the *hyomandibular* cleft. The others are simply numbered (second, third, and fourth). Above the mandibular arch the rudiment of the *trigeminal* ganglion may be seen; above the hyoid arch, the rudiment of the *acustico-facialis* in contact with the anterior walls of the auditory sac; above the third visceral arch, the rudiment of the ganglion of the glossopharyngeus; and above the fourth and fifth, the rudiment of the *vagus* ganglion. Observe the form and position of the heart. In the trunk observe the appearance of the anterior and posterior limb-rudiments. How many mesoblastic somites are there? Make a careful drawing of the entire mount.

3. *Sections.*—Sections transverse to the trunk pass about horizontally through the hind-brain, and transversely through the fore-brain (*cf.* drawing of entire mount). The plane of the sections should be indicated by lines across the drawing of the entire embryo; this will be found greatly to facilitate the study of the sections of

the head. Embryos of this age must be studied principally by systems of organs, though the following representative sections should be drawn first:

a) Through the hind-brain, showing the *auditory sacs,* the *neuromeres,* and the *trigeminal acustico-facialis glossopharyngeus* and *vagus ganglia.*

b) Through the upper portion of the pharynx, showing mid-brain, hind-brain, visceral pouches, etc. Identify nerves and blood-vessels.

c) Through the choroid fissure of the optic cups. Draw the lens under a high power also. On the other side the section will pass through the heart.

d) Through the olfactory pits.

e) Through the hepatic and pancreatic diverticula. Identify the parts.

f) Through the hind-gut and the beginning of the allantois.

4. *Study of systems of organs.*

a) *The alimentary tract and its appendages.* —Note the way in which the *splanchnopleure* folds to form the walls of the intestine. How is the *mesentery* established? The most anterior part of the alimentary tract is formed from the ectoderm (*stomodæal invagination.*) A dorsal outgrowth of this forms the *hypophysis.* What

is its relation to the brain? The following outgrowths of the entoderm are found in embryos of this stage: (1) *Visceral pouches.* Note the *visceral furrows* in the ectoderm opposite each. Is the closing membrane ruptured? (2) The median rudiment of the *thyroid,* an outgrowth of the flood of the pharynx between the two hyoid arches. (3) *Lung-rudiments,* paired outgrowths of a median ventral diverticulum of the alimentary tract, just behind the last visceral pouch. The *œsophagus* is just posterior to this. It is very short at this stage, and is continuous with a slightly wider part that develops into the *stomach.* (4) The *first liver diverticulum,* the origin of which from the intestine may be found a few sections posterior. The second liver-diverticulum is found immediately behind. Just back of this, at the point where the intestine opens ventrally, the *pancreas* (5) is indicated by a slight thickening of the dorsal angle of the intestine. From this place to the hind-gut there are no other outgrowths, though the *yolk-sac* (6) is an appendage of the alimentary tract. The ventral wall of the hind-gut forms a wide evagination, (7) the beginning of the *allantois.*

Make an outline sketch of the whole embryo, and in this reconstruct the alimentary canal.

b) *The heart and blood-vessels.*—The ar-

rangement of the chambers of the heart and of the principal blood-vessels is essentially the same in a three-day chick as in a fish.

(1) The heart is still a simple tube, though differences in the relations, size, and shape of different sections clearly mark off the following divisions: *sinus venosus, auricular portion, ventricular portion,* and *bulbus arterosus.* The *sinus venosus* is formed by the union of the two *ductus Cuvieri* and the *ductus venosus.* These in their turn are formed as follows: the ducts of Cuvier by the union of the *anterior* and *posterior cardinal veins* on each side, the ductus venosus by the union of the right (smaller) and left (larger) *vitelline veins* that return the blood from the yolk-sac. The sinus venosus is the most dorsal portion of the heart, and is situated immediately above the single *auricle* into which it opens. The latter is very wide laterally, indicating its future division in two. The auricle opens directly into the *ventricle,* practically all of which lies behind and ventral to the auricle, owing to the bending of the heart (see models). The ventricle is therefore the first part of the heart to appear in a series of transverse sections studied from behind forward; its most posterior portion (angle of bend) represents the future apex of the heart. The *bulbus arteriosus* (continuation

forward of ventricle) lies immediately beneath the auricular portion of the heart, and divides shortly into a number of *aortic arches,* a pair ascending in each pair of visceral arches.

All of these points may easily be verified from the sections.

(2) The aortic arches unite above the visceral arches to form the roots of the dorsal aorta, which are continued anteriorly for a short distance as the *carotid* arteries. The dorsal aorta passes back beneath the notochord, and after dividing into two parallel dorsal aortae gives off on each side the vitelline artery. What other branches of the dorsal aorta are found in this stage?

(3) *Veins.*—Find the anterior and posterior cardinals, the vitelline veins, the ductus venosus and the duct of Cuvier. See (*b*) (1).

In an outline of the embryo reconstruct the circulatory system, showing by arrows the direction of the blood-flow.

c) *The nervous system.*—The general anatomy of the central nervous system has already been observed. The character of the walls in each division of the brain and in the spinal cord should be carefully noted; also the form of the central canal and its various divisions (ventricles and passages) in the brain. Are the cells

composing the walls of the neural tube arranged in more than one layer? In what part of the wall are dividing nuclei found? What is the significance of this?

As concerns the peripheral nervous system, it will be sufficient to note here only a few facts, because the development of the nerves proper is hardly begun. The dorsal and ventral roots of the spinal nerves arise separately and unite secondarily. The ventral roots grow out at regular intervals from *neuroblasts* within the cord. The dorsal roots, on the other hand, arise from the spinal ganglia, which are segmental collections of neuroblasts formed from the *neural crest.* This may be observed in the stage of 48 hours, as a tongue of cells arising on each side from the angle between the neural tube and the external epiblast. In the stage of 72 hours neither the dorsal nor the ventral roots, as such, are likely to be observed, but at least the rudiments of the spinal ganglia may be seen. In the head the neural crest forms four primary ganglia, viz., the ganglia of the *trigeminus* over the mandibular arch, of the *acustico-facialis* over the hyoid, of the *glossopharyngeus* over the third visceral arch, and of the *vagus* over the third and fourth visceral clefts. Of these the first two are clearly seen in the stage under consideration.

Reconstruct the system as in a) and b).

d) The excretory system (*mesonephros*). The *pronephros* is not well developed in the chick.

Mesonephros (Wolffian body).—This consists primarily of a series of tubules imbedded in the mesenchyme lateral to the dorsal aorta on each side, and opening into the *Wolffian duct* that lies just beneath the cardinal vein.

(1) *The Wolffian duct.*—How far forward does this extend? Posteriorly? Does it open into the cloaca? Describe its position in earlier stages.

(2) *Tubules.*—Each consists of a blind, dilated, thin-walled portion, situated near the median portion of the Wolffian body and the tubule proper that passes transversely to open into the duct. The upper wall of the thin-walled part is invaginated by a mass of mesenchyme that receives a small vessel from the dorsal aorta. Thus the *Malpighian corpuscle* with its *glomerulus* and *capsule* of *Bowman* is established. These parts are more distinctly differentiated in the four-day chick.

How many tubules to a segment? By study of earlier stages can you trace them back to the *intermediate cell mass?*

What is the antero-posterior extent of the Wolffian body?

V. STUDY OF AN EMBRYO OF ABOUT NINETY-SIX HOURS

1. *Living embryo.*—How much of the yolk is now covered by the blastoderm? Note any changes since the seventy-second hour. Draw to scale. Kill as before and preserve.

2. Entire stained and cleared mounts of this stage are sufficient only for the general topography. The hardened embryo should also be studied in alcohol with the dissecting microscope, and the following observations made:

a) The embryo now lies in a space (extra-embryonic body cavity) bounded below by the *splanchnopleure* and above by the *chorion* (*false amnion* or *serosa*). Is the latter attached at any point to—

b) The *amnion* that closely invests the embryo? Determine this point by carefully stripping off the chorion.

c) Carefully remove the amnion from around the embryo. What relation has it to the *somatic umbilicus?* What is the relation of the splanchnopleure and *splanchnic umbilicus?* What relation has the *allantois* to the parts already mentioned?

d) *The head.*—Identify the parts of the brain. Do you find the *pineal gland* (*epiphysis*)? Do the *cerebral hemispheres* appear enlarged since the seventy-second hour? Note changes

in the olfactory pit. In the *eye* observe the *lens* and *choroid fissure* (reason for this name?). The *maxillary process* of the *mandibular arch* lies beneath the eye and behind the olfactory pits. What is the relation of the other *arches* and *clefts?* Is the otocyst visible? Where?

e) *The trunk.*—Position and condition of limb rudiments? Heart? Wolffian ridges? Allantois? Tail?

Make a drawing *or model in clay* of the embryo studied from the side.

Cut off the head carefully just behind the last visceral arch. Study and draw it from the ventral surface. See especially *nasal pits, maxillary processes, mandibular* and *hyoid arches.* The *fronto-nasal process* is just appearing at this stage.[3]

3. *Study of transverse sections.*—Cut transversely to the trunk. The series will not be understood unless the curvature of the various parts of the embryo is carefully taken into account. Refer constantly to the drawing or model of the entire embryo in studying the sections.

a) *Draw a section through the anterior limb-rudiments under the low power.*—Identify all parts. Pay special attention to *spinal ganglia,*

[3] See FOSTER AND BALFOUR, *op. cit.*, p 201.

muscle plate (*myotome*), condensation of mesenchyme around notochord, *excretory organs, intestine, liver, pancreas,* distribution of mesenchyme. Study all of these parts under the high power. What is the meaning of the condensation around the notochord? Do you find the *ventral roots* of the nerves? Can you distinguish *neuroblasts* in the cord? In the ganglia?

b) *Study of the nervous system and sense-organs.*—The first sections pass horizontally through the hind- and mid-brain regions. Draw one of these, including the *auditory vesicle.* Identify the parts. In neighboring sections find the ganglia of the *trigeminus, acustico-facialis, glossopharyngeus,* and *pneumogastric nerves.*

From here follow the series along. Observe the appearance of the notochord and anterior cardinal veins. Keep the rudiments of the above-mentioned nerves in view as long as possible through the series. What becomes of them? As we pass farther along, the ear disappears, and we have the mid-brain at one end of the section, and the cord at the other. By referring to the drawing of the entire embryo, it will be seen that the region between must lie just above the pharynx. Look therefore for the appearance of the visceral arches. Do not fail to observe the *third cranial nerve* (oculo-motor) arising from

the floor of the mid-brain. A good many other structures begin to appear, which may for the present be neglected.

As the series passes from the mid-brain to the thalamencephalon, the *infundibulum* begins to appear as a ventral prolongation of the floor of the latter. Beneath the infundibulum is a small tube, the *hypophysis*. Follow the latter, and observe that it opens into the mouth. It is an ingrowth of the oral epithelium. Draw a section showing thalamencephalon, infundibulum, and hypophysis. Draw it all in outline, but leave the trunk part to be completed later.

The next section selected for drawing should be through the center of the eyes and should include, if possible, the *lens*, *optic stalk*, and the region of the optic chiasma. Find the *choroid fissure*. Dram a median section of the lens under *the high power* also. The *pineal gland* (epiphysis) begins to appear beyond the eyes. Observe and describe its relations and structure. The rudiments of the cerebral hemispheres (telencephalon) lie a little farther forward. Draw a section showing their relations to the thalamencephalon. Finally, draw a section through the olfactory pits.

c) *Study of the alimentary tract and its appendages* (*outgrowths*).

The mouth and pharynx.—At this stage the mouth is bounded by the mandibular arches, maxillary processes, and ventral surface of the head. The double membrane formerly separating it from the pharynx has ruptured. A finger-shaped diverticulum of its roof grows out toward the infundibulum and forms the *hypophysis.* The form of the pharynx is rather complex, owing to the great development of the visceral pouches. Remembering that the sections are about horizontal in direction through the pharynx, identify and label in the drawings already made, the various visceral pouches and arches. Do any of the visceral pouches actually communicate with the exterior? Do all of them? Do you find an artery in each arch? The *thyroid* diverticulum may already have lost its communication with the pharynx; but it can be found in the thickened floor, and shows evidence of budding.

The *lung-rudiments* have changed greatly. Distinguish *glottis* (opening at the most posterior portion of the pharynx or beginning of œsophagus), long unpaired *trachea,* paired *bronchi.* What is the extent of the various parts? Draw sections illustrating different regions, and make a reconstruction.

The *liver* has already become a large organ. It surrounds the common trunk of the vitelline

veins, which it divides in two parts: a part nearer the heart, called the *sinus venosus;* and a part surrounded by the liver; which is called the *ductus venosus.*[4] Draw a section through the liver, showing the ductus venosus. Draw a portion of the liver under the high power. How is the present condition derived from that seen in the 72 hour chick?

The *stomach* is indicated by a considerable dilatation situated above the tip of the ventricle. Immediately behind the stomach the *hepatic duct* (bile duct) may be found; this is formed by partial fusion of the original right and left ducts.[5]

The *pancreas* is much further developed. Find and describe. Trace the intestine farther back. Find the yolk-stalk (*splanchnic umbilicus*). Draw a section though this. Find the stalk of the *allantois,* and trace its connection with the hind-gut. Draw a part of the wall of the allantois under the high power.

d) *Study of the urinogenital system.*—The following parts of the urinogenital system are found in the 96 hour embryo: the *Wolffian ducts,* the beginning of the *Müllerian duct,* the *mesonephros* (embryonic kidney), the *germinal epithelium,* the beginning of the duct of the

[4] See FOSTER AND BALFOUR, *op. cit.*, pp. 226–28, for further details concerning the blood supply of the liver.

[5] *Ibid.*, pp. 178–81.

metanephros (permanent kidney). These are all (except the last) constituent parts of the *urinogenital ridge* (derived from the nephrotome), which forms a rounded projection on each side of the mesentery into the dorsal angles of the body-cavity.

(1) The *Wolffian ducts,* one on each side, begin far forward and may be traced back along the lateral margin of the ridge to the cloaca, into which they open. Identify them in the first section drawn. Trace them through their entire length. Draw the section showing the openings into the cloaca. Observe the openings of numerous tubules into them along the greater part of their length.

(2) The *Müllerian ducts* arise from a thickened strip of epithelium situated along the lateral surface of the urinogenital ridge, beginning near its anterior end. Identify this. Show it in one of the drawings. Trace it as far as possible. Read the account in Foster and Balfour.

(3) The *mesonephros* forms the greater part of the ridge. It consists of a series of tubules, in each of which two parts may be distinguished: the *Malpighian corpuscle,* consisting of a thin-walled *capsule* invaginated by a tuft of capillaries from the aorta forming the *glomerulus,* and the *tubule proper,* that leads from the capsule to the

Wolffian duct. Work out the relation of these parts. Construct a diagram of an entire tubule from about the middle of the mesonephros, showing the relations of all parts and the blood-supply.

(4) The *germinal epithelium* is the rudiment of the essential portions of the *gonad* (ovary or testis). It is a thickening of the peritoneum of the median wall of the ridge. It is found near the anterior end of the ridge. Look for "primitive ova" in it. Show its position in a drawing.

(5) Duct of the *metanephros* (*ureter*). This arises as a dorsal diverticulum of each *Wolffian duct* near the posterior termination of the latter. It is difficult to work out its relations in the transverse series, owing to the curvature of the embryo at this place. It will be seen better in the sagittal series of sections.

e) *Heart and circulation.*—The general arrangement is the same as on the third day. Follow the account given in Foster and Balfour.[6] Identify and label the blood-vessels shown in the sections already drawn.

4. *Study of sagittal sections.*—Study of a series of sagittal sections serves to bring out many relations much more clearly than a transverse series, especially in the anatomy of the head and posterior regions of the body.

[6] *Op. cit.*, pp. 224 ff.

a) The more lateral sections pass through the eye transversely to its axis. The nature of the choroid fissure is brought out especially well. Draw a section through the eye. Passing toward the median line, the side of the brain is cut. Sections should be drawn to show the relations of the fifth, seventh, ninth, and tenth nerves to the visceral arches.

b) Draw a median section through the head showing the parts of the brain and including the *epiphysis, infundibulum, hypophysis,* mouth, and pharynx.

c) The relations of the blood-vessels should also be worked out and illustrated.

d) Sections through the muscle-plates show the longitudinal course of the muscle-fibres. *Draw.* The same section should show the position of the spinal ganglia.

e) Work out in the same series, the relations of the *thyroid body,* the *lungs,* and the *liver.*

f) Study the sections of the posterior end, and determine the following points: (1) relation of yolk-stalk and allantois to the intestine; (2) relation of the *Wolffian ducts* to the intestine; (3) origin of the *ureters* from the Wolffian ducts; (4) Is the anus formed? Can you determine its subsequent position?

PART II

Study of Pig Embryos

STUDY OF PIG EMBRYOS

I. DISSECTION OF THE UTERUS AND FŒTAL MEMBRANES OF THE PIG.

1. In the fresh specimen note the shape and type of the uterus. Follow one horn towards its distal end; note the position and attachment of the *broad ligament*. The end of the uterus is rounded, and continuous on one side with a much smaller, strong, dense tube, the *Fallopian tube,* which is much convoluted and ends in a thin-walled sac surrounding the ovary, except for a terminal aperture into the body-cavity, the *ostium tubæ abdominale.*

2. Examine the ovary, its size, shape, position, color, etc. Note the large colored *corpora lutea* of the recent pregnancy, and the immature *Graaffian follicles.* Count the corpora lutea in both ovaries. How does the number compare with the number of embryos in the uterus indicated by the series of enlargements in the two horns?

3. Make a drawing of the uterus and its parts.

4. Take a formalin-hardened specimen, wash in water, and dissect under water. With fine scissors cut away the wall of one of the enlargements of the uterus opposite to the ligament,

removing at the same time the outer embryonic covering.

5. Identify the *muscular layer* and *mucosa* of the wall of the uterus. Note the position of the blood-vessels, and the *outermost of the embryonic membranes, the chorion,* and its relation to the uterine wall. (The chorion in the pig in composed of the chorion proper and the wall of the allantois.)

6. The inner surface of the uterus is thickly beset with ridges running transversely, and largest in the center of each compartment. In life the pressure of the fluids enclosed by the chorion keeps it and the uterine wall in close contact, thereby holding the embryo in place. This is known as *diffuse placentation.*

7. The *embryo* surrounded by another thin semi-transparent membrane, the *amnion,* is attached to the chorion in the region of the larger ridge by a stalk, the *umbilical cord,* and thus floats in the fluid-filled cavity of the chorion. The embryo is thus surrounded by two membranes, the *amnion* and the *chorion,* and two fluid-filled cavities, the *amniotic* and the *allantoic.* (At this stage the cavity of the chorion has become obliterated by the growth of the allantois, so that the apparent cavity of the chorion is the allantoic cavity.) The *yolk-sac* is a large sac flattened between the allantois and chorion,

attached to the intestine of the embryo by the *yolk-stalk* running through the umbilical cord.

8. Observe the structure of the *umbilical cord.* Note the blood-vessels in it and trace their distribution in the allantois. What is the relation of the chorion to the umbilical cord in the region of the large uterine ridge?

Make a drawing of the uterus cut open, showing the parts found and their relation to one another.

9. Sever the umbilical cord as far from the embryo as posible, and study the latter in a watch-glass under the dissecting microscope.

10. *The amnion*—Are blood-vessels present? What is the relation of the amnion to the umbilical cord? to the body wall? to the chorion?

11. Make a diagram showing the relations of the embryo, embryonic membranes, the cavities, and the wall of the uterus. Describe.

II. EXTERNAL ANATOMY AND GROSS DISSECTION OF PIG EMBRYOS

1. *Pig embryo of about 10 mm. neck-length.—Study in alcohol with the dissecting microscope.*[7]

[7] Figures both of the entire embryo and of sections will be found in MINOT's *Laboratory Text Book;* see also KIEBEL, *Normaltafeln zur Entwicklungsgeschichte der Wirbelthiere,* I, Figs. 17, 18, 19.

a) *Lateral view.*—What is the exact length of your embryo? Measure from the cervical flexure to the posterior convexity (neck-length). Carefully strip off the amnion. What is its relation to the body of the embryo and the umbilical cord?

In the head distinguish *cranial* and *cervical flexures.* Compare these with the seventy-two or ninety-six hour chick. Determine the position of the hind-brain, mid-brain, fore-brain, and the cerebral vesicles. Compare the *eye* with that of the three- or four-day chick. Find the *olfactory pits.* Behind the eye observe the *maxillary process* of the *mandibular arch.* The *hyoid arch* is large. The third, fourth, and fifth visceral arches are hidden in the *cervical sinus.* Of the visceral clefts only the first or *hyomandibular* (the rudiment of the auditory meatus, middle ear, and Eustachian tube) is visible. In embryos of 5 mm. neck-length the four visceral clefts are fully exposed. In the stage of 7 mm. the posterior cleft begins to be covered up by the formation of a pocket-like fold of the integument open in front. In the 8 and 9 mm. stages this closes more and more until only the first visceral cleft is left uncovered. Thus the cervical sinus is formed.

In the trunk observe the *umbilical* cord, the

rudiments of the limbs, the external molding of the mesoblastic somites, the *milk-ridge* (rudiment of the mammary glands) extending between the bases of fore and hind limbs, and the tail. The postanal portion of the body (tail) tapers gradually and is not sharply marked off from the trunk. The knob immediately in front of the tail on the ventral surface is the *genital tubercle.* The following internal organs affect the surface contours of the sides and ventral region of the body: the *heart,* the *Wolffian body,* the *liver,* and the *embryonic diaphragm.* Make an enlarged drawing or model (x 10) to scale of your embryo from one side. What are the principal external differences at this stage between the avian and mammalian embryo?

b) *The embryo may readily be dissected* under alcohol. Strip off the lateral body-wall and remove the limbs. Thus the heart, liver, diaphragm, Wolffian body, and intestine are laid bare. The latter forms a loop descending deep into the umbilical cord; at the bottom of the loop is the *yolk-stalk* that can readily be traced into the *yolk-sac.*

Draw the dissection.

c) *Remove the head* of a 10 mm. pig by an oblique cut behind the cervical sinus. Study with the dissecting microscope.

(1) The mouth is bounded behind by the *mandibular arches.* The antero-external boundary on each side is formed by the *maxillary process,* which meets the *nasofrontal process,* bounding the mouth in front, at a groove (*lachrymal groove*) extending down and inwards from the eye.

(2) The olfactory pits occur near the lateral margins of the nasofrontal process. Each is bounded laterally by the *lateral frontal process,* and medially by the *globular process* of the *median frontal process.* The nasal pits are not clearly separated from the mouth-cavity on their inferior aspect.

(3) The cerebral hemispheres project very slightly in front of the nasofrontal process.

Make a drawing of the head from in front.

2. *Pig embryo of about 15 mm. neck-length.* —Are the flexures as pronounced as before? The *cervical sinus* has closed up, and the last trace of the third to the fifth visceral arches thus disappears from the surface. The hyomandibular cleft is more prominent than before. Describe its position and the modification of the bounding arches. The eye is pigmented. Describe the changes in the limbs since the last stage. What is the condition of the milk-ridge? Note changes in the form of the *genital tubercle*

since the stage of 10 mm. Dissect as in the preceding stage. Compare the heart, liver, Wolffian body, and intestine with the 10 mm. stage. *Draw.*

Development of the face.—Remove the head and examine it from all sides. Can you still distinguish the nasofrontal process, the maxillary processes, the lachrymal groove, etc? Is the upper jaw complete? Describe the way in which the snout is formed. Draw the head from in front.

3. *Pig embryo of about 25 mm. neck-length.*—This embryo is distinctly recognizable as a young pig. Compare the cranial and cervical flexures with earlier stages. Describe the *external auditory meatus* (which visceral cleft?), the *external ear* (from which arch?) the *eye-lids.* Observe the beginnings of the large sensory hairs.

Describe the changes in the limbs since the preceding stage. Is the tail definitely developed? What changes in the milk-ridge? *Draw from the side.* Amputate the head. Describe the progress in the development of the snout. Are the elements of the face still distinguishable? Draw the head.

Dissect as before. Compare the relative importance of the Wolffian body. Find the per-

manent kidney. The lungs are readily found. Note the diaphragm and its relations to heart and liver.

III. DETAILED STUDY OF A 10 MM. EMBRYO.[8]

1. *Transverse series of sections.—Cut these parallel to the line of the hind-brain. Use great care in orienting.*

Rule lines across the drawing of the entire embryo parallel to the plane of the sections. This will be found to aid greatly in studying the sections.

Most of the organs are in a more advanced stage of development than those of the 96 hour chick. But some, especially the eye, appear less advanced.

a) Select for the first drawing a section a short distance back of the rudiments of the anterior limbs. This section should pass through the stomach, liver, and tip of the heart. The neural tube, the notochord, stomach, Wolffian bodies, liver, and heart will be readily recognized. Confirm the following details, and include them in the drawing (which should be not less than three inches in diameter):

(1) Each spinal nerve arises from two *roots, dorsal* and *ventral,* the former of which bears a

[8] A good description of the anatomy of this embryo is given by MINOT in his *Laboratory Text-Book.*

large *ganglion.* The *neuroblasts* of the dorsal root are found in the ganglion; of the ventral root, in the ventro-lateral portion of the cord,

(2) Each spinal nerve divides into three main branches (*a*) the *dorsal* branch passing dorsally just external to the ganglion; (*b*) the *ventral* branch passing into the somatopleure; (*c*) the *visceral* branch (*ramus communicans*) passing toward the aorta from the dorsal angle of the body-cavity.

(3) The *notochord* is extremely small, and is surrounded by a dense accumulation of mesenchyme, the beginning of the *vertebral axis.*

(4) The *somatopleure* consists of three layers: *ectoderm, mesenchyme,* and *mesothelium.* The last is the *peritoneal lining* of the body-cavity, and it may be traced completely around the body-cavity, being everywhere adherent to the body-wall or the viscera.

(5) The *muscle-plate* lies external to the spinal nerve. It is lightly stained, the differentiation of fibers having already begun.

(6) *Adventitious coats* are beginning to form around the original endothelial wall of the *aorta.*

(7) Distinguish the endodermal and mesenchymal layers of the wall of the stomach. The latter is very thick.

(8) The *dorsal mesentery* of the stomach (*mesogaster,* rudiment of *great omentum*) is bent over to the left.

(9) Note the *gastro-hepatic ligament* (rudiment of the *lesser omentum*).

(10) The small cœlomic space to the right of the stomach is the rudiment of the cavity of the omentum.

(11) The *Wolffian bodies* are extremely large. Their *glomeruli* are on the median aspects. The *duct* is near the ventral angle of each.

(12) The liver has two lobes on each side. It is attached ventrally to the somatopleure, and so divides the body-cavity in two lateral parts. Its substance is broken up into cords of cells by numerous blood-sinuses.

b) *Nervous system and sense organs.*

(1) The ear: Describe the *membranous labyrinth.* Find the *endolymphatic duct.* Draw. Find *external auditory meatus, tympanum, Eustachian tube, middle ear.*

(2) The ganglia of the cranial nerves: What are they? Do you find the eleventh and twelfth nerves?

(3) The *neuromeres* of the hind-brain.

(4) Into what visceral arches do the fifth to the tenth cranial nerves enter?

(5) The *infundibulum, epiphysis, cerebral*

hemispheres.

(6) The eye: Do you find the beginnings of the *cornea, vitreous humor, arteria centralis, retinæ*? Draw.

(7) The nose (*olfactory pit*).

(8) *Spinal nerves:* Relations of ganglion to dorsal root? Branches of the nerve? Draw.

(9) Draw a section of the cord showing dorsal and ventral zones of His, neuroblasts, etc.

c) *Alimentary tract.*—The same divisions are found as in the 72 or 96 hour chick. Illustrate by drawings the *visceral pouches*, the *trachea, bronchi, lungs, liver, pancreas, yolk-stalk, allantois.* The *gall-bladder* is forming; a loop of the intestine extends into the beginning of the umbilical cord. The duct of the median rudiment of the thyroid gland is closed.

d) *The visceral arches.*—Each contains a nerve and an artery. In each arch, what is the derivation of these? Observe the appearance of the *cervical sinus* in this section.

e) *Urinogenital system.*—No sign of the Müllerian duct in the pig at this stage. Otherwise the same parts are present as in the 96 hour chick. The mesonephros is very large. What is its extent (antero-posterior)? Work out the relations of glomeruli and tubules. What is the blood-supply of the glomeruli? Find the open-

ings of the Wolffian ducts into the cloaca. Do you find the beginnings of the metanephric ducts? The dilations of these form the rudiments of the pelvis of the kidney. Better seen in sagittal sections. The germinal epithelium is not very well marked. Are there any indications of separation of the anal from the urinogenital opening?

f) *Heart and blood-vessels.*—The position of the heart at this stage is such that sections cut in the plane indicated pass obliquely across its long axis. The stage of development of the heart is transitional toward complete double circulation. The structure of the heart and the relations of the blood-vessels are therefore especially interesting.

(1) Draw a section showing the communication of auricular and ventricular divisions Note auricular and vetricular septa, valves, apertures.

(2) Study the *auricles* in their whole extent. Can you distinguish the right auricle from the left? How? Is the auricular septum everywhere complete? What name is given to the foramen?[9]

(3) Does the *sinus venosus* still constitute a separate chamber?

(4) Study the *ventricles* in their whole ex-

[9] See McMurrich's *Embryology*. Compare figures of development in this, and also models of embryonic heart.

tent. Compare the condition of the septum with that of the auricular septum.

(5) Is the *bulbus arteriosus* also partially subdivided?

(6) Illustrate points under (2)-(5) by drawings.

(7) *The arterial system.*—What aortic arches exist in this stage? What arteries are found in the head? In the trunk? *Make a diagram of the arterial system.*

(8) *The venous system.*—Do you find any trace of the *pulmonary veins?* What veins enter the right auricle? What veins pass through the liver? Find the *allantoic* vein. Describe its course. *Make a diagram of the venous system.*

2. *Sagittal series of sections.*

a) *Description of an approximately median sagittal section of a ten millimeter pig embryo.*—A section exactly median in all its extent is something never realized in actual experience. The description will therefore include some structures not in the median line. It should be remembered that no single section can include all the details mentioned below.

(1) *Parts of the nervous system.—Identify telencephalon, thalam, (or di-) encephalon, mesencephalon, metencephalon (or cerebellum),* and *myelencephalon (or medulla oblongata).*

Take particular note of the thin roof of the last. What are the ridges in its floor? The *hypophysis* should be noted and its exact relation to the mouth and brain carefully noted in the drawing. The *infundibulum* is not yet formed, though the region of the brain from which it arises is plainly marked.

In the region of the cord the section is likely at some place to pass to one side of it. The great size of the spinal ganglia should be noted in this place.

(2) *Parts of the alimentary canal.*—The mouth, pharynx, œsophagus, and part of the stomach appear in a median section. In the floor of the pharynx the median rudiment of the *thyroid body* may be observed. The opening of the *glottis* appears at the hinder end of the embryonic pharynx. The long *trachea* may be traced beneath the œsophagus to the place of bifurcation. The intestine is not likely to fall in the plane of this section; but part of the *cloaca* is pretty sure to be included. The stalk of the allantois or the opening of one of the Wolffian ducts may be found. The position of the *liver* and its fusion with the ventral body-wall just in front of the umbilicus should be noted. The *pancreas* lies in the dorsal mesentery just behind the stomach.

(3) *Heart and blood-vessels.*—Identify the parts of the heart included. *Note the valves.* The connection of the pericardial with the general pleuroperitoneal cavity should be noted. Parts of the *dorsal* and *ventral aorta,* of the *umbilical veins,* the *vitelline arteries* and *veins,* the *allantoic artery,* and other vessels may be found.

(4) The origin of the ureter from the subterminal portion of the Wolffian duct may be shown.

(5) If the section passes far enough to one side in any part, study the *muscle plates* under the high power. Observe that the muscle fibers run longitudinally.

(6) *The notochord.*—Where does it end anteriorly? Note its wavy course and slight diameter. Explain the appearance of segmentation in the dense mesenchyme surrounding it.

Make a drawing of such a section about six inches long.

b) *Study of system of organs.*—Any system of organs may be studied in the sagittal series and special directions are not needed if the study of the transverse series has been at all thorough. From the study of such a series make the following drawings and reconstruction:

(1) *Plan of the cloaca,* including the relations of *intestine, Wolffian ducts, metanephric*

ducts, allantois, anus, urinogenital aperture, and *relation of all to the genital tubercle.*

(2) Drawing through the center of a myotome.

(3) Plan of the intestine and its appendages.

[redacted]